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RESEARCH ARTICLE

FTIR-assisted evaluation of xanthan gum as a biostimulant for groundnut seed priming and growth promotion

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Abstract

Experiments were conducted to evaluate the effect of biostimulant-based seed priming on the germination and seedling vigour of groundnut (*Arachis hypogaea*) seeds. Seeds with 75 % initial germination and 9 % moisture content were primed using xanthan gum, carrageenan, gellan gum, chitosan and gelatin at five concentrations (0.1 %-0.5 %), two soaking volumes (equal and double volume) and three soaking durations (1-3 hr). Seed quality parameters were assessed under both normal and stress conditions. The results indicated that seeds primed with 0.2 % xanthan gum (double volume, 1 hr) showed the highest germination (100 %), root length (16.20 cm), shoot length (8.00 cm) and vigour index (2420). These results were statistically similar to the 0.3 % xanthan gum treatment (double volume, 2 hours). The next best performance was observed with 0.3 % gellan gum (double volume, 1 hr), resulting in 100 % germination, 15 cm root length, 8.30 cm shoot length and a vigour index of 2335. In contrast, untreated control seeds displayed lower performance. The top five priming treatments were further evaluated under water stress conditions (60 %, 70 %, 80 % and 100 % water-holding capacity of sand). Seeds primed with 0.2 % xanthan gum (double volume, 1 hour) achieved the highest germination rates under all stress levels (60 %-100 %), whereas control seeds recorded lowest values. FTIR analysis of xanthan and gellan gum identified key functional groups associated with improved nutrient uptake, stress resistance and enhanced metabolic processes-factors contributing to increased plant growth in primed seeds. Biostimulant seed priming, particularly with xanthan and gellan gum, significantly enhances seed germination, vigour and stress tolerance in groundnut, suggesting a promising pre-sowing treatment for improved productivity in both irrigated and rainfed conditions.

Keywords: biostimulants; FTIR analysis; groundnut; seed germination; seed priming; seedling vigour; water stress

Introduction

India's population has grown significantly, from 1.22 billion in 2009 to approximately 1.42 billion in 2022. As a result, Indian agriculture faces the simultaneous challenge of enhancing productivity to meet the food demands of the growing population while efficiently utilizing available resources. An estimate suggests that approximately 6 billion people worldwide could be affected by water scarcity by 2050 (1). Agricultural inputs such as fertilizers and pesticides are playing a vital role in improving crop yields. However, their continuous and over usage leads to deterioration of soil structure, soil fertility and destroy the biodiversity in agricultural environments and productivity.

Groundnut (*Arachis hypogaea*) is the fourth most important oilseed crop worldwide, with a global 42.4 million tonnes (2). Major producers include China, India, Nigeria, Senegal, Sudan, Burma and the United States. India ranks second globally, producing 6 million tonnes from 5.5 million hectares. Most of the groundnut cultivation in India occurs in the states of Gujarat, Andhra Pradesh, Tamil Nadu and Karnataka. In Tamil Nadu alone, groundnut is cultivated in an area of 3.72 lakh hectares, yielding 1.047 million tonnes at an average productivity of 2200 Kg/ha. Notably, about 85 % of India's groundnut is sown during the *kharif* season under rainfed conditions (3). Conventional approaches such as chemical fertilizers and pesticides have been adopted to ensure maximum food production. However, the indispensable input of these chemical products creates a global concern due to their harmful impacts on environment. It is a high time to focus on enhancing the productivity of rainfed crops by using the quality seeds and adopting technological interventions to mitigate water stress and to expand groundnut cultivation in dry tracks. Several innovations have been proposed to enhance the sustainability in agriculture through a significant reduction of synthetic agrochemicals. One promising strategy involves the use of natural plant biostimulants (PBS). These substances enhance plant physiological processes, promoting growth, flowering, fruit set, nutrient uptake and stress tolerance. Biostimulants can be defined as substances or microorganisms-or combinations thereof-that, when applied to plants, seeds, or the rhizosphere, improve nutrient efficiency, crop quality, yield and resilience to abiotic stress, without acting as direct fertilizers or pesticides (4).

Xanthan gum (XG), a high-molecular-weight polysaccharide produced by *Xanthomonas campestris*, has been widely utilized in agriculture for applications such as hydrogels, mulch films and controlled-release carriers (5). However, systematic investigations into its role as a biostimulant in seed treatment are limited. Seed coating offers an efficient method to apply biostimulants, minimizing the required quantity while reducing labor and field application costs.

Fourier Transform Infrared (FTIR) analysis identifies the functional groups present in the biostimulants, which offers a detailed understanding of how biostimulant treatments influence the chemical composition and metabolic processes, thus enhancing our understanding of the underlying mechanisms that improve seed performance. Nevertheless studies on seed treatments with such a biostimulants for improving the seed vigour and productivity are very limited. In this context, the present study was designed to assess the effectiveness of various natural biostimulants, particularly xanthan gum, on seed germination, vigour and productivity in groundnut. Additionally, FTIR analysis was employed to characterize the functional groups responsible for the observed biological effects (6, 7).

Materials and Methods

Truthfully labelled seeds (TFL) of groundnut *(Arachis hypogaea* L.) variety VRI 10, with a germination percentage of 75 % and moisture content of 9 %, were collected from the Agricultural College and Research Institute, Kudumiyanmalai, Pudhukottai, Tamil Nadu and used as the base material for the study.

Seed priming treatments

Groundnut seeds were subjected to priming with five different natural biostimulant namely xanthan gum, carrageenan, gellan gum, chitosan and gelatin. Each biostimulant was tested at five concentrations: 0.1 %, 0.2 %, 0.3 %, 0.4 % and 0.5 %. For each concentration, two soaking volumes were used (equal volume and double the seed volume), along with three soaking durations (1-3 hr). The experiment followed a completely randomized design (CRD) with multiple replications per treatment. Post-priming, the seeds were shade-dried and subjected to further evaluation.

Germination test

Germination tests were conducted in a sand medium using four replications of 25 seeds each. The tests were carried out in a germination room under controlled conditions at 25 ± 2 ° C and 95 ± 3 % relative humidity. The number of normal seedlings was counted on the 10th day and the mean germination percentage was calculated as per standard procedures (8).

Shoot length (cm)

The shoot length of all normal seedlings was measured from the collar region to the shoot apex and expressed in cm.

Root length (cm)

The root length of all normal seedlings was measured from the collar region to the root tip and expressed in cm.

Vigour index (I)

Vigour index values were calculated using the formula proposed by Abdul Baki and Anderson (9), with the mean values expressed as whole numbers.

Vigour Index = Germination (%) × Total Seedling Length (cm)

Vigour index (II)

Vigour index values were calculated using the formula proposed by Abdul Baki and Anderson (9), with the mean values expressed as whole numbers.

Vigour Index (II) =

Germination (%) × Dry Matter Production (g/10 seedlings)

The most effective treatments were selected for further studies.

Moisture stress evaluation

To assess drought tolerance, selected priming treatments were evaluated under varying levels of soil moisture: 60 %, 70 %, 80 % and 100 % of the water-holding capacity (WHC) of sand. Germination percentage was recorded and compared with unprimed control seeds.

FTIR spectral analysis of xanthan and gellan gum

Fourier Transform Infrared (FTIR) analysis was conducted to identify the functional groups in xanthan gum and gellan gum. The analysis was carried out at the Anbil Dharmalingam Agricultural College and Research Institute, Tiruchirappalli, Tamil Nadu. Approximately 2 mg of each powdered sample was mixed with 200 mg of potassium bromide (KBr) and pressed into a pellet. The pellet was placed in the FTIR sample holder and spectra were recorded in the 4000 to 400 cm^{-1} range. The characteristic absorption peaks were identified and compared with reference spectra to determine the functional groups responsible for bioactivity.

Statistical analysis

Data from all experiments were statistically analyzed using Analysis of Variance (ANOVA) using R to assess significant differences between treatments. The F-test of significance was applied following the methods outlined by Rangaswamy (10). When necessary, percentage values were transformed into angular (arcsine) values before analysis. Critical differences (CD) were calculated at 5 % and 1 % probability levels. Statistical significance was determined and if the F test was non-significant, it was denoted by the letters “NS.”

Results and Discussion

A significant difference was observed in biostimulant seed priming on seed germination, seedling vigour and stress tolerance in groundnut. Five biostimulants-xanthan gum, carrageenan, gellan gum, chitosan and gelatin-were evaluated for their impact on seed quality parameters under laboratory conditions. Among them, xanthan gum and gellan gum exhibited the highest values across all measured parameters, including germination rate, root length, shoot length and vigour index, as outlined below.

Germination %

The results showed that groundnut seeds primed in double the volume of 0.2 % xanthan gum for 1 hr achieved the highest germination rate (100 %), which was comparable to seeds primed in double the volume of 0.3 % gellan gum for 1 hr (100 %) and those primed in an equal volume of 0.4 % gelatin for 1 hr (100 %). The lowest germination rate (75 %) was recorded in the control group (Fig. 1).

Shoot length (cm)

Groundnut seeds primed in the double the volume of 0.2 % xanthan gum for 1 hr recorded the highest shoot length of (8.00 cm), which was on par with double the volume of 0.3 % gellan gum primed for 1 hr (8.30). The least value was recorded in control (6.59).

Root length (cm)

Same trend was recorded in groundnut seeds primed in the double the volume of 0.2 % xanthan gum for 1 hr recorded the highest root length of (16.20 cm), which was on par with double the volume of 0.3 % Gellan gum primed for 1 hr (15.00 cm). The least values were recorded in control (13.70 cm).

Vigour index

The highest vigour index (2420) was recorded in seeds primed with 0.2 % xanthan gum (double volume, 1 hr), followed by 0.3 % gellan gum (2335) and 0.4 % gelatin. The control group recorded the lowest vigour index (Fig. 2). The xanthan gum enhances seed hydration and seedling growth, likely due to its gel-like matrix that improves water retention in black gram and ragi, these findings are consistent with the studies (11, 12). Furthermore, xanthan gum has been investigated for biochar encapsulation, offering an innovative method to enhance organic fertilization and nutrient uptake. This indicates that its use as an encapsulating agent could be a valuable approach to extending the effectiveness of biostimulants under field conditions (13).

Similarly, gellan gum promoted seedling growth, with treated seeds exhibiting 100 % germination and significant root and shoot lengths of 15 cm and 8.30 cm, respectively. However, xanthan gum-treated seeds consistently outperformed gellan gum-treated seeds in terms of vigour index, with xanthan gum yielding the highest value (2420). This could be attributed to its enhanced water-retention capacity and its ability to solubilize nutrients, which are critical during early seedling development (14).

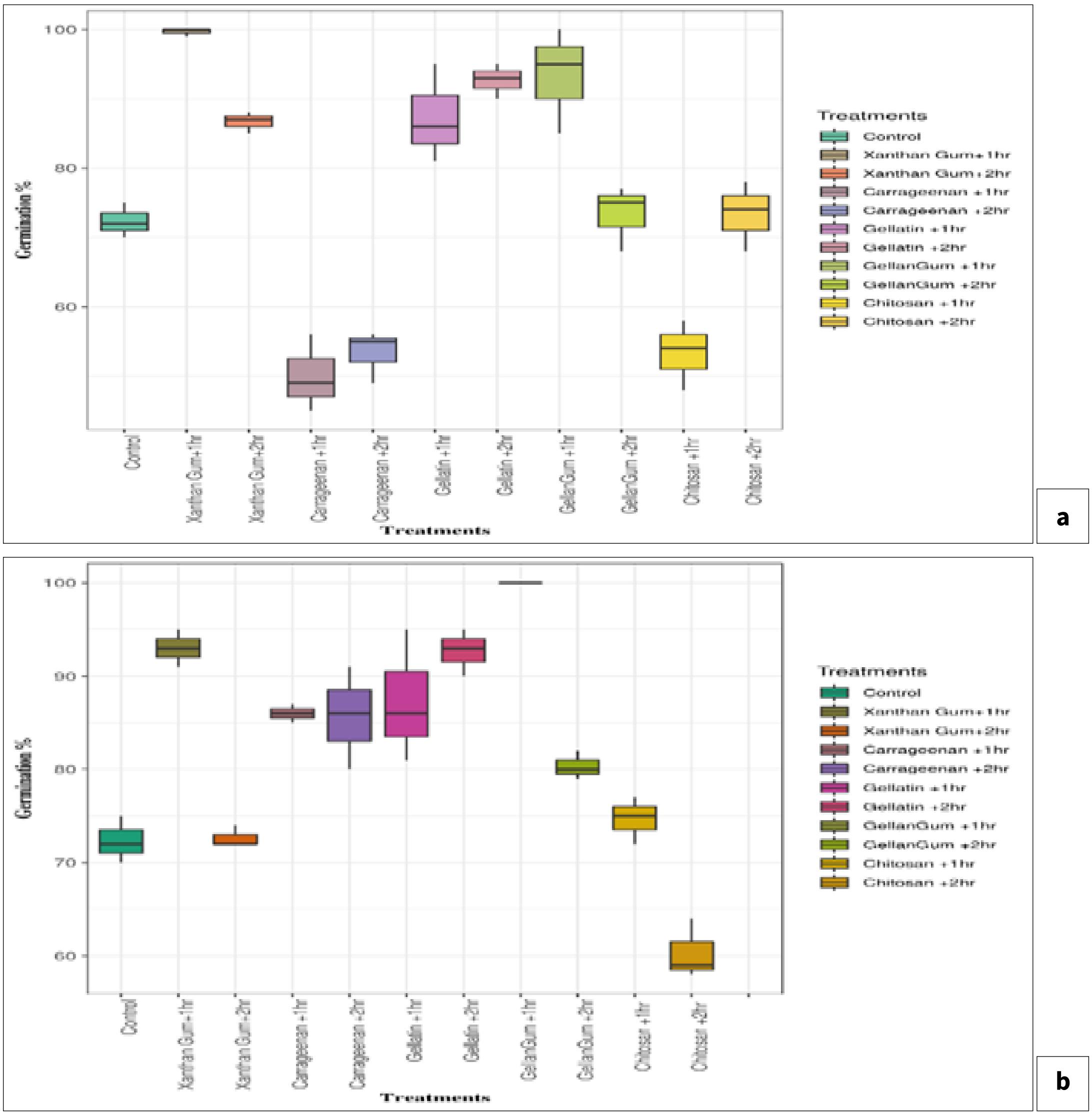

Fig. 1. (a) Effect of biostimulant seed priming on germination percentage of groundnut at 0.2 % concentrations. (b) Effect of biostimulant seed priming on germination percentage of groundnut at 0.3% concentrations. Grouping is based on the LSD test. Treatments with the same letters do not differ significantly at the 5 % level.

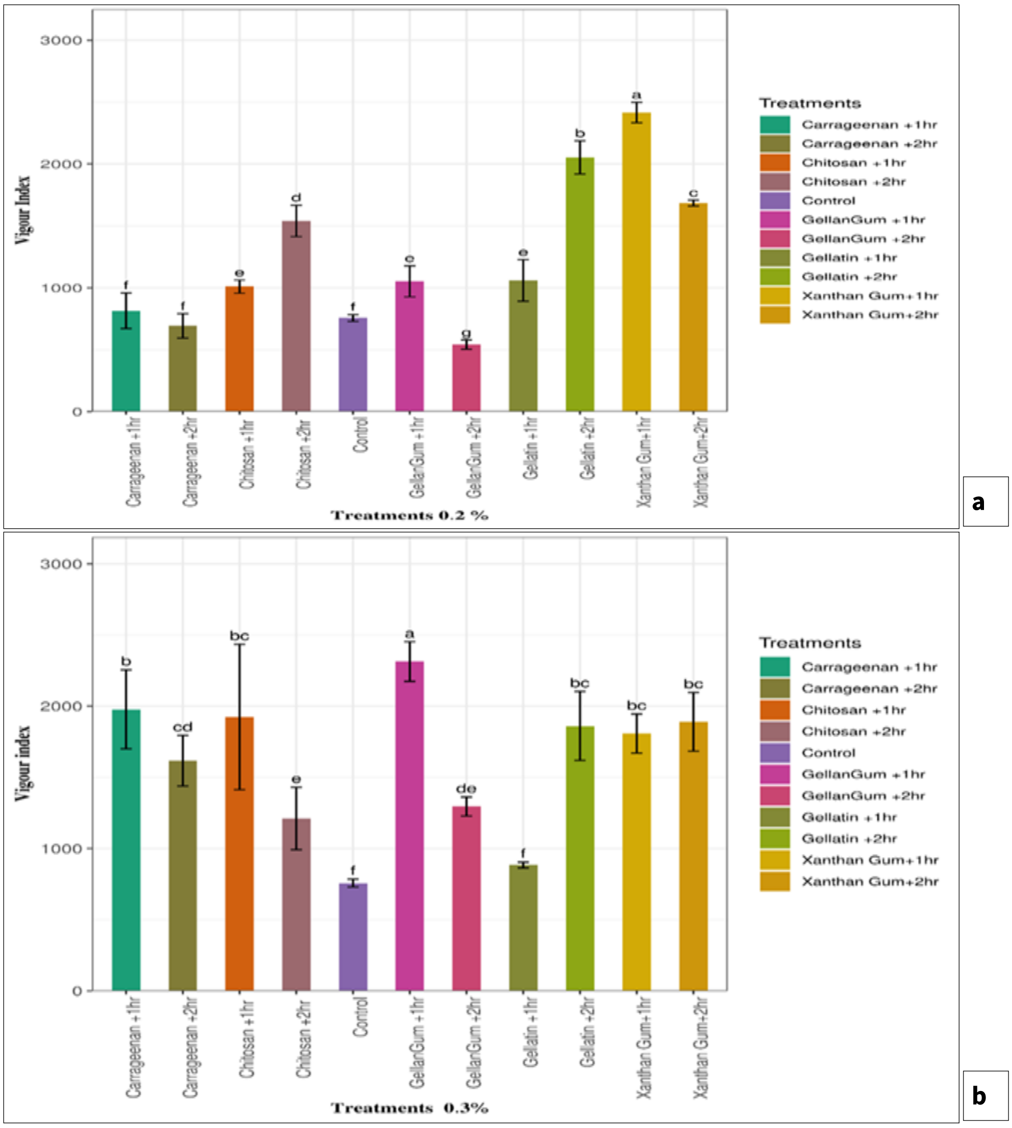

Fig. 2. (a) Effect of biostimulant seed priming on vigour of groundnut at 0.2 % concentrations. (b) Effect of biostimulant seed priming on vigour of groundnut at 0.3 % concentrations. Grouping is based on the LSD test. Treatments with the same letters do not differ significantly at the 5 % level.

Seed germination under water stress conditions

The seed priming treatments were further evaluated under water stress conditions and results indicated that xanthan gum-treated seeds exhibited superior drought tolerance. Under various water-holding capacities (60 %, 70 %, 80 % and 100 % WHC), seeds primed with 0.2 % xanthan gum for 1 hr showed the highest germination rates (78 %, 80 %, 85 % and 100 %), whereas control seeds demonstrated much lower germination percentages (50 %, 60 %, 75 % and 78 %) under similar conditions (Fig. 3). This indicates that xanthan gum priming can significantly enhance seed resilience under drought stress, likely due to its water -holding properties and the improvement of seedling metabolic processes (15). It's gel -forming nature may help in preserving membrane stability and enzyme activity, thereby enhancing seedling survival and establishment (16).

FTIR analysis of xanthan gum and gellan gum

FTIR spectral analysis of xanthan gum and gellan gum reveals key insights into their chemical composition and helps explain their effectiveness as biostimulants in promoting plant growth and stress resilience. The FTIR spectrum of xanthan gum (Fig. 3, Table 1) identified several functional groups relevant to plant growth. A prominent O-H stretching vibration at 3260.92 cm^{-1} corresponds to alcohol groups, while C-H stretch at 2823.26 cm^{-1} and 2883.15 cm^{-1} indicate the presence of alkanes. A C-N stretching vibration at 1252.97 cm^{-1} reflects the presence of amines, which play roles in nitrogen metabolism and protein synthesis. The C-O stretch (1020.63 cm^{-1}) in xanthan gum, associated with ethers, alcohols and carboxylic acids, suggests its role in enhancing nutrient solubility and uptake, which is crucial for seedling growth. The aromatic C=C stretch (1601.07 cm^{-1}) points to the potential involvement of secondary metabolites in defense mechanisms, helping seedlings with stand environmental stresses (17). Overall, the functional groups in xanthan gum support its role in improving nutrient uptake, stress resistance and cell wall integrity, all of which are essential for promoting seedling vigour and resilience under water stress.

FTIR analysis of gellan gum (Fig. 4, Table 2) also identified biologically significant functional groups. The P=O stretching at 1025.41 cm^{-1} and P-O bending at 890.74 cm^{-1} are indicative of phosphate groups, which are essential for energy transfer, nucleic acid function and root development (13). Aromatic compounds (C=C stretch at 1598.93 cm^{-1}) in gellan gum may play a role in secondary metabolism and plant defense, while C-H stretches (2822.53 cm^{-1} and 2882.84 cm^{-1}) highlight the presence of alkanes and aldehydes, which

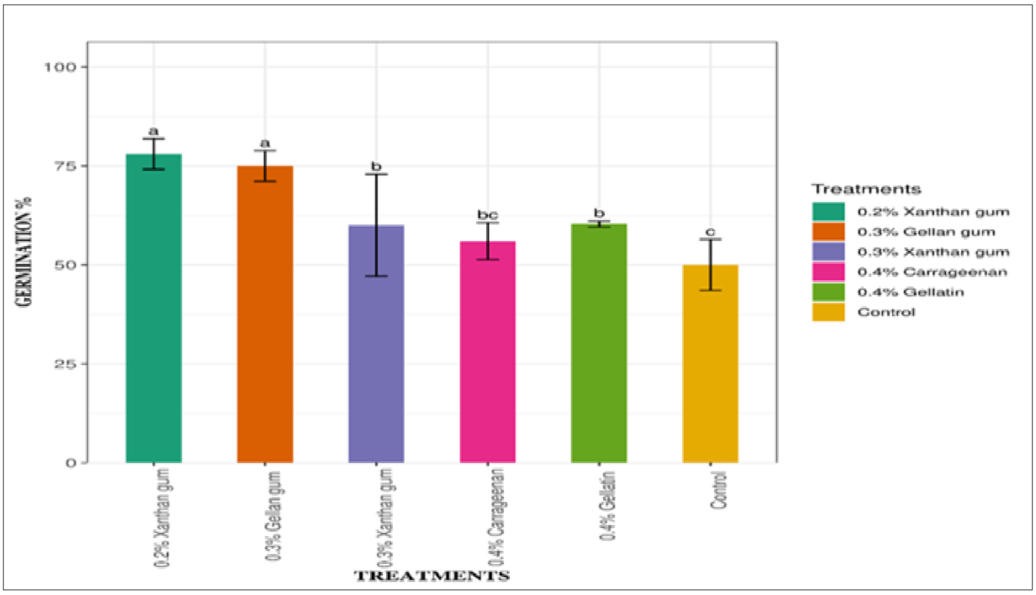

Fig. 3. Effect of biostimulant seeds priming on moisture stress tolerance in groundnut under the different water holding capacities. Grouping is based on the LSD test. Treatments with the same letters do not differ significantly at the 5 % level.

Table 1. FT-IR absorption frequencies (cm^{-1}), intensity and functional group of Xanthan gum and their role in plant growth promotion

Wave number (cm^{-1})	Intensity	Possible Functional Group	Bond Type	Role in Plant Growth Promotion
1020.63	68.856	C-O stretch (ethers, alcohols, carboxylic acids)	C-O	Enhances nutrient solubility and uptake, supports cell wall formation (25)
1252.97	91.180	C-N stretch (amines) or asymmetric SO_2 stretch (sulfones)	C-N / S=O	Amines contribute to protein synthesis and enzyme activity; sulfones aid in stress response
1403.91	89.70	C-H bending (methyl or methylene groups)	CH_3 / CH_2	Structural component of plant lipids and secondary metabolites
1601.07	85.44	C=C stretch (aromatic or alkene) or N-H bend (amines)	C=C / N-H	Aromatic rings are essential for lignin and flavonoid synthesis, aiding defense mechanisms
2823.26	99.25	C-H stretch (aldehyde)	C-H	Aldehydes participate in hormone signaling and secondary metabolism
2883.15	98.84	C-H stretch (alkanes)	C-H	Provides hydrophobic properties essential for membrane stability
3260.92	91.83	O-H stretch (alcohols, phenols) or N-H stretch (amines, amides)	O-H / N-H	Important for antioxidant activity, stress resistance and signaling molecules

Table 2. FT-IR absorption frequencies (cm^{-1}), intensity and functional group of gellan gum and their role in plant growth promotion

Wave number (cm^{-1})	Intensity	Possible Functional Group / Vibration Mode	Role in Plant Growth Promotion
890.74	91.222	P-O bending (phosphates) or C-H out-of-plane bending (aromatic compounds)	Phosphates are crucial for energy transfer (ATP), DNA/ RNA function and root development. Aromatic compounds are involved in plant defense, UV protection and secondary metabolism
1025.41	76.881	P=O stretching (inorganic phosphate groups)	Phosphorus is essential for metabolism, phosphorylation reactions and cell division. It supports energy transfer, genetic material function and growth regulation
1403.61	93.208	C-H bending (alkanes) or symmetric NO_2 stretching (nitro compounds)	Alkanes are part of cuticle waxes, protecting against water loss and pathogens. Nitro compounds (less common) may indicate environmental
1598.93	90.764	C=C stretching (aromatic compounds, alkenes) or N-H bending (amines)	Aromatic compounds are involved in secondary metabolism, UV protection and pollinator attraction. Amines (e.g., amino acids) are essential for protein synthesis and cell growth
2323.65	101.680	Possibly CO_2 overtones or other stretching modes	$CO_2$is the main source of carbon for photosynthesis, which is essential for energy production, growth and metabolic processes in plants
2822.53	100.716	C-H symmetric stretching (alkanes, aldehydes)	Alkanes are found in cuticle waxes protecting plant surfaces. Aldehydes are involved in plant stress responses, including oxidative stress and wound healing
2882.84	100.368	C-H asymmetric stretching (alkanes, CH_3 groups)	Alkanes contribute to cuticle waxes, providing waterproofing and protection against pathogens. CH_3 groups are found in alkanes and influence plant metabolic processes
3257.93	95.454	N-H stretching (for amines) or O-H stretching (for alcohols and phenols)	Amines are involved in protein synthesis and nitrogen metabolism. Alcohols and phenols are critical for cell wall synthesis, defense mechanisms and stress responses

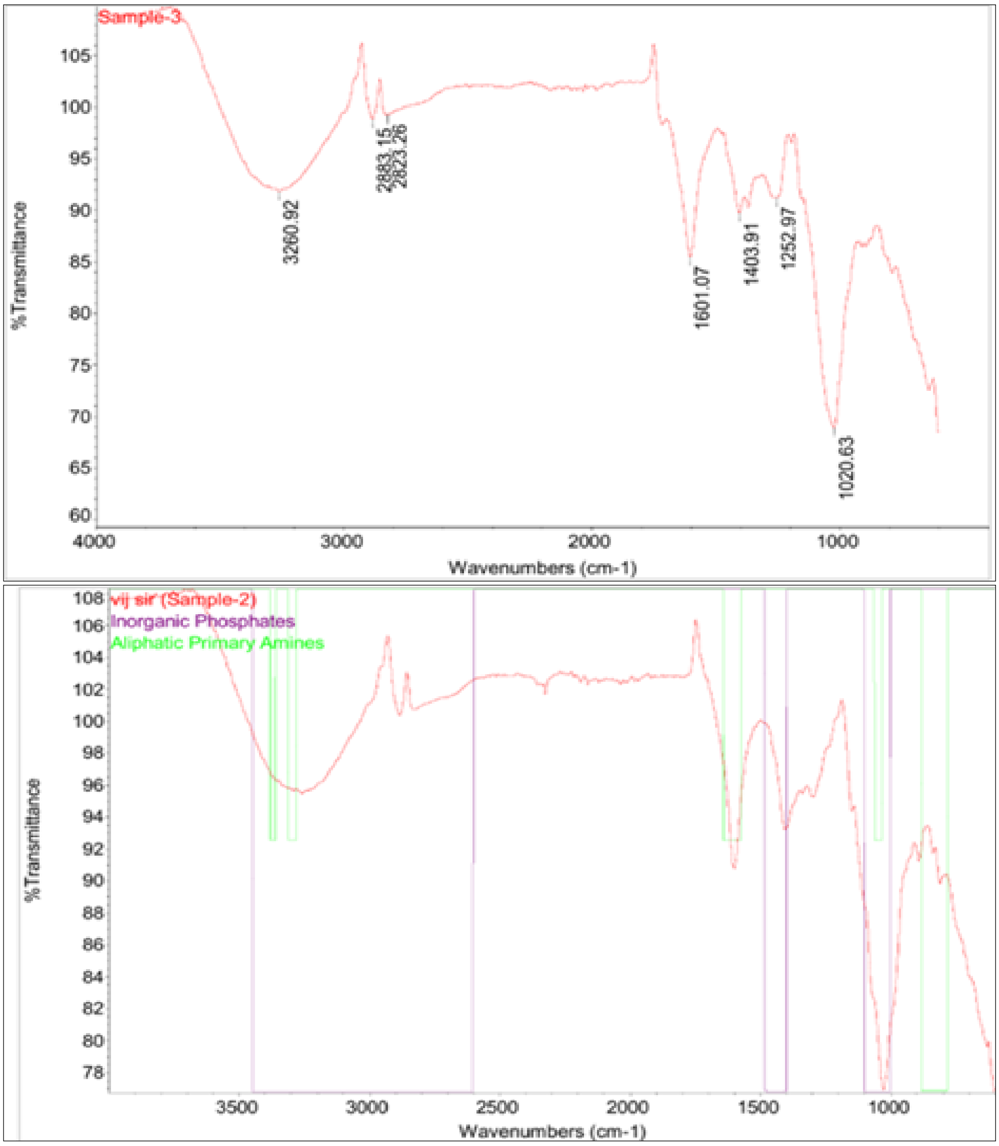

Fig. 4. FT-IR spectrum of (a) xanthan gum and (b) gellan gum.

contribute to stress responses and membrane stability (18). Additionally, N-H stretching (3257.93 cm^{-1}) indicates the presence of amines, which are involved in protein synthesis and nitrogen metabolism, both vital for overall plant growth and stress tolerance (19).

Both xanthan gum and gellan gum exhibit shared functional groups, such as alcohols and amines, which are important for promoting seedling growth and stress resilience. However, differences in the presence of phosphate groups and aromatic components suggest divergent modes of action. Xanthan gum appears to enhance nutrient solubility and water retention, while gellan gum may promote root development and metabolic activity through phosphate-associated mechanisms (20).

The identified functional groups contribute to improved seed germination, seedling vigour and enhanced resilience under water stress conditions. These findings, combined with experimental data, underscore the potential of biostimulant seed priming as a sustainable agricultural practice to enhance crop productivity, especially in rainfed areas where water scarcity is a significant challenge (21-23).

This study discovered that biostimulants like xanthan gum and gellan gum provide a viable, eco-friendly substitute for synthetic fertilizers and pesticides, promoting the development of sustainable agricultural practices in response to climate change and drought stress (24). Based on the studies, it could be concluded that pre-sowing treatment of groundnut seeds by priming them with 0.2 % xanthan gum in double the volume of water for one hour or 0.3 % gellan gum is recommended to enhance seed vigour and boost productivity in groundnut cultivation.

Conclusion

Biostimulant seed priming with xanthan gum and gellan gum significantly enhanced groundnut seed germination, seedling vigour and stress tolerance. The most effective treatment was 0.2 % xanthan gum (double volume for 1 hr), achieving 100 % germination and the highest vigour index. Under water stress, primed seeds showed superior germination compared to untreated seeds. FTIR analysis confirmed the presence of functional groups that improve nutrient uptake, stress resistance and metabolic processes, making biostimulant priming a promising pre-sowing treatment for improved seed quality and productivity.

Authors' contributions

GM carried out the research work. VV involved in seed priming Standardization & FTIR studies. SS involved in statistical analysis. NS involved in technical writing. VM involved in water stress studies. GG involved in statistical analysis. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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